

Claims

What is claimed is:

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- 5 1. An isolated polypeptide having phospholipase B activity, selected from the group consisting of:
 - (a) a polypeptide having an amino acid sequence which has at least 65% identity with amino acids 20 to 464 for the mature polypeptide of SEQ ID NO. 2;
 - 10 (b) a polypeptide which is encoded by a nucleic acid sequence which hybridizes under low stringency conditions with (i) nucleotides 568 to 2045 of SEQ ID NO. 1, (ii) the cDNA sequence contained in nucleotides 568 to 2045 of SEQ ID NO. 1, (iii) a subsequence of (i) or (ii) of at least 100 nucleotides, or (iv) a complementary strand of (i), (ii), or (iii);
 - (c) a variant of the polypeptide having an amino acid sequence of SEQ ID NO. 2 comprising a substitution, deletion, and/or insertion of one or more amino acids;
 - 15 (d) an allelic variant of (a) or (b); and
 - (e) a fragment of (a), (b), or (d) that has phospholipase B activity.
- 20 2. The polypeptide of claim 1, having an amino acid sequence which has at least 65% identity with amino acids 20 to 464 of SEQ ID NO. 2.
3. The polypeptide of claim 2, having an amino acid sequence which has at least 70% identity with amino acids 20 to 464 of SEQ ID NO. 2.
4. The polypeptide of claim 3, having an amino acid sequence which has at least 80% identity with amino acids 20 to 464 of SEQ ID NO. 2.
- 25 5. The polypeptide of claim 4, having an amino acid sequence which has at least 90% identity with amino acids 20 to 464 of SEQ ID NO. 2.
- 30 6. The polypeptide of claim 5, having an amino acid sequence which has at least 95% identity with amino acids 20 to 464 of SEQ ID NO. 2.

7. The polypeptide of any of claims 1-6, comprising the amino acid sequence of SEQ ID NO. 2.
- 5 8. The polypeptide of any of claims 1-7, consisting of the amino acid sequence of SEQ ID NO. 2 or a fragment thereof.
9. The polypeptide of claim 8, consisting of the amino acid sequence of SEQ ID NO. 2.
- 10 10. The polypeptide of claim 9, which consists of amino acids 20 to 464 of SEQ ID NO. 2.
11. The polypeptide of claim 1, which is encoded by a nucleic acid sequence which hybridizes under low stringency conditions with (i) nucleotides 568 to 2045 of SEQ ID NO. 1, (ii) the cDNA sequence contained in nucleotides 568 to 2045 of SEQ ID NO. 1, (iii) a subsequence of (i) or (ii) of at least 100 nucleotides, or (iv) a complementary strand of (i), (ii), or (iii).
- 15 12. The polypeptide of claim 11, which is encoded by a nucleic acid sequence which hybridizes under low stringency conditions with (i) nucleotides 568 to 2045 of SEQ ID NO. 1, (ii) the cDNA sequence contained in nucleotides 568 to 2045 of SEQ ID NO. 1, or (iii) a complementary strand of (i) or (ii).
- 20 13. The polypeptide of claim 1, which is encoded by a nucleic acid sequence which hybridizes under medium stringency conditions with (i) nucleotides 568 to 2045 of SEQ ID NO. 1, (ii) the cDNA sequence contained in nucleotides 568 to 2045 of SEQ ID NO. 1, (iii) a subsequence of (i) or (ii) of at least 100 nucleotides, or (iv) a complementary strand of (i), (ii), or (iii).
- 25 14. The polypeptide of claim 13, which is encoded by a nucleic acid sequence which hybridizes under medium stringency conditions with (i) nucleotides 568 to 2045 of SEQ ID
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NO. 1, (ii) the cDNA sequence contained in nucleotides 568 to 2045 of SEQ ID NO. 1, or (iii) a complementary strand of (i) or (ii).

15. The polypeptide of claim 1, which is encoded by a nucleic acid sequence which hybridizes under high stringency conditions with (i) nucleotides 568 to 2045 of SEQ ID NO. 1, (ii) the cDNA sequence contained in nucleotides 568 to 2045 of SEQ ID NO. 1, (iii) a subsequence of (i) or (ii) of at least 100 nucleotides, or (iv) a complementary strand of (i), (ii), or (iii).

16. The polypeptide of claim 15, which is encoded by a nucleic acid sequence which hybridizes under high stringency conditions with (i) nucleotides 568 to 2045 of SEQ ID NO. 1, (ii) the cDNA sequence contained in nucleotides 568 to 2045 of SEQ ID NO. 1, or (iii) a complementary strand of (i) or (ii).

17. The polypeptide of claim 1, wherein the polypeptide is a variant of the polypeptide having an amino acid sequence of SEQ ID NO. 2 comprising a substitution, deletion, and/or insertion of one or more amino acids.

18. The polypeptide of claim 1, which is encoded by the nucleic acid sequence contained in plasmid pPH6 which is contained in *E. coli* NRRL B-30142.

19. The polypeptide of any of claims 1-18 which has at least 20% of the phospholipase B activity of SEQ ID NO. 2.

20. A polypeptide having the same phospholipase B activity as the polypeptide of any of claims 1-19.

21. An isolated nucleic acid sequence comprising a nucleic acid sequence which encodes the polypeptide of any of claims 1-20.

22. An isolated nucleic acid sequence comprising a nucleic acid sequence having at least

one mutation in the mature polypeptide coding sequence of SEQ ID NO. 1, in which the mutant nucleic acid sequence encodes a polypeptide consisting of amino acids 20 to 464 of SEQ ID NO. 2.

23. An isolated nucleic acid sequence produced by (a) hybridizing a DNA under low stringency conditions with (i) nucleotides 568 to 2045 of SEQ ID NO. 1, (ii) the cDNA sequence contained in nucleotides 568 to 2045 of SEQ ID NO. 1, (iii) a subsequence of (i) or (ii) of at least 100 nucleotides, or (iv) a complementary strand of (i), (ii), or (iii); and (b) isolating the nucleic acid sequence.

24. The isolated nucleic acid sequence of claim 23 produced by (a) hybridizing a DNA under medium stringency conditions with (i) nucleotides 568 to 2045 of SEQ ID NO. 1, (ii) the cDNA sequence contained in nucleotides 568 to 2045 of SEQ ID NO. 1, (iii) a subsequence of (i) or (ii) of at least 100 nucleotides, or (iv) a complementary strand of (i), (ii), or (iii); and (b) isolating the nucleic acid sequence.

25. An isolated nucleic acid sequence of claim 24 produced by (a) hybridizing a DNA under high stringency conditions with (i) nucleotides 568 to 2045 of SEQ ID NO. 1, (ii) the cDNA sequence contained in nucleotides 568 to 2045 of SEQ ID NO. 1, (iii) a subsequence of (i) or (ii) of at least 100 nucleotides, or (iv) a complementary strand of (i), (ii), or (iii); and (b) isolating the nucleic acid sequence.

26. A nucleic acid construct comprising the nucleic acid sequence of claim 21 operably linked to one or more control sequences that direct the production of the polypeptide in a suitable expression host.

27. A recombinant expression vector comprising the nucleic acid construct of claim 26.

28. A recombinant host cell comprising the nucleic acid construct of claim 26.

29. A method for producing a mutant nucleic acid sequence, comprising (a) introducing at

least one mutation into the mature polypeptide coding sequence of SEQ ID NO. 1, wherein the mutant nucleic acid sequence encodes a polypeptide consisting of amino acids 20 to 464 of SEQ ID NO. 2; and (b) recovering the mutant nucleic acid sequence.

5 30. A mutant nucleic acid sequence produced by the method of claim 29.

31. A method for producing a polypeptide, comprising (a) cultivating a strain comprising the mutant nucleic acid sequence of claim 30 encoding the polypeptide to produce a supernatant comprising the polypeptide; and (b) recovering the polypeptide.

10 32. A method for producing the polypeptide of any of claims 1-20 comprising (a) cultivating a strain to produce a supernatant comprising the polypeptide; and (b) recovering the polypeptide.

15 33. A method for producing the polypeptide of any of claims 1-20 comprising (a) cultivating a host cell comprising a nucleic acid construct comprising a nucleic acid sequence encoding the polypeptide under conditions suitable for production of the polypeptide; and (b) recovering the polypeptide.

20 34. A method for producing a polypeptide comprising (a) cultivating a host cell under conditions conducive for production of the polypeptide, wherein the host cell comprises a mutant nucleic acid sequence having at least one mutation in the mature polypeptide coding sequence of SEQ ID NO. 1, wherein the mutant nucleic acid sequence encodes a polypeptide consisting of amino acids 20 to 464 of SEQ ID NO. 2, and (b) recovering the polypeptide.

25 35. A method for producing the polypeptide of any of claims 1-20 comprising (a) cultivating a homologously recombinant cell, having incorporated therein a new transcription unit comprising a regulatory sequence, an exon, and/or a splice donor site operably linked to a second exon of an endogenous nucleic acid sequence encoding the polypeptide, under
30 conditions conducive for production of the polypeptide; and (b) recovering the polypeptide.

36. A method for producing a mutant of a cell, which comprises disrupting or deleting a nucleic acid sequence encoding the polypeptide of any of claims 1-20 or a control sequence thereof, which results in the mutant producing less of the polypeptide than the cell.

37. A mutant produced by the method of claim 36.

38. The mutant of claim 37, which further comprises a nucleic acid sequence encoding a heterologous protein.

39. A method for producing a heterologous polypeptide comprising (a) cultivating the mutant of claim 38 under conditions conducive for production of the polypeptide; and (b) recovering the polypeptide.

40. A nucleic acid construct comprising a gene encoding a protein operably linked to a nucleic acid sequence encoding a signal peptide consisting of nucleotides 510 to 567 of SEQ ID NO. 1, wherein the gene is foreign to the nucleic acid sequence.

41. A recombinant expression vector comprising the nucleic acid construct of claim 40.

42. A recombinant host cell comprising the nucleic acid construct of claim 40.

43. A method for producing a protein comprising (a) cultivating the recombinant host cell of claim 42 under conditions suitable for production of the protein; and (b) recovering the protein.

44. A method for reducing the phospholipid content in an edible oil, comprising treating the oil with the polypeptide of claim 1 to hydrolyze the phospholipid and separating the hydrolyzed phospholipid from the oil.

45. A method for reducing the phospholipid content of an aqueous carbohydrate solution or slurry, comprising treating the aqueous carbohydrate solution or slurry with the

polypeptide of claim 1 to hydrolyze the phospholipid and separating the hydrolyzed phospholipid from the aqueous carbohydrate solution or slurry.

46. A method for preparing a dough, comprising incorporating into the dough an effective amount of the polypeptide of claim 1 to improve one or more properties of the dough and/or a baked product obtained from the dough.

47. The method of claim 46, wherein the one or more improved properties are selected from the group consisting of increased strength of the dough, increased stability of the dough, reduced stickiness of the dough, improved machinability of the dough, increased volume of the baked product, improved crumb structure of the baked product, improved softness of the baked product, improved flavor of the baked product, and improved antistaling of the baked product.

48. A method for preparing a baked product, comprising baking a dough produced by the method of claim 46 or 47 to produce a baked product.

49. A dough product obtained from a dough prepared by the method of claim 46 or 47.

50. A baked product produced by the method of claim 48.

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